Status of the Pubertal Assays

December 2005

Endocrine Disruptor Methods Validation Advisory Committee meeting

Purpose and outline of the talk

The purpose of this status report is to refresh EDMVAC's memory about the general outline of the pubertal validation effort. Data and results of specific studies are not included as they will be discussed at the next meeting. The presentation is informational; comments are not being solicited.

- Description of the assays (design, endpoints)
- Studies done in the past (optimization, etc.)
- Issues raised at EDMVS meetings; EPA responses
- Current studies (interlaboratory validation)
- Plans for the next EDMVAC meeting

Description of female pubertal

Design:

- 15 females per dose level
- 2 dose levels* + control
- Gavage daily in corn oil, postnatal days (PND) 22 through 42
- Daily check for vaginal opening (VO) from PND22 until complete
- Daily check for stage of estrous cycle after VO

Endpoints:

- Growth (body weight)
- Organ weights (uterus, ovaries, liver, kidneys, pituitary, adrenals)
- Histology (uterus, ovary, thyroid)
- Hormone levels (T_4, TSH)

*MTD and ¼ MTD, where MTD is defined as ≤10% decrease in final body weight between treated and controls if no other appropriate guidance is available.

Description of male pubertal

Design:

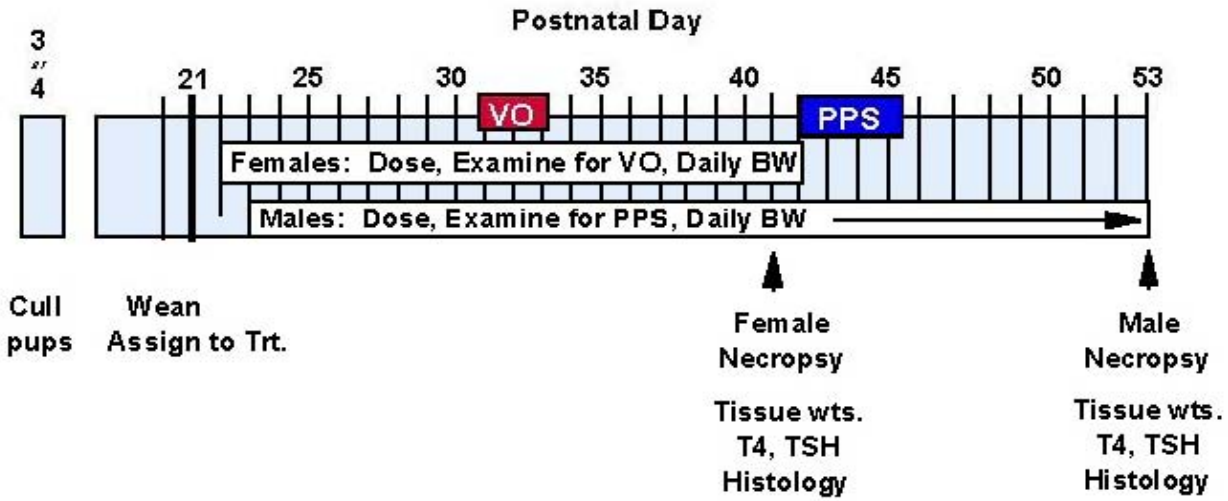
- 15 males per dose level
- 2 dose levels* + control
- Gavage daily in corn oil, postnatal days (PND) 23 through 53
- Daily check for preputial separation (PPS) from PND30 until complete

Endpoints:

- Growth (body weight)
- Organ weights (testes, epididymides, seminal vesicles, ventral prostate, dorsolateral prostate, levator ani/bulbocavernosus muscle complex, liver, kidneys, pituitary, adrenals)
- Histology (testis, epididymis, thyroid)
- Hormone levels (T_4, TSH, testosterone)

*MTD and ¼ MTD, where MTD is defined as ≤10% decrease in final body weight between treated and controls if no other appropriate guidance is available.

Schematics for female and male pubertal assays



Past studies

- Single-dose-level study (TherImmune 2000)

  Tested the performance of the assay across a variety of chemicals at a single high dose level when run in a contract laboratory.

- Multi-chemical study (RTI 2003)

  Tested a larger variety of chemicals in a contract laboratory, at two dose-levels each.

- Multi-Dose-Level study (TherImmune 2003)

  Tested sensitivity of the assays in a contract laboratory (3 dose levels for each of 3 chemicals).

Issues raised at EDMVS meetings, and EPA responses

- Dose selection

  Issue: Should dose-selection be considered part of the assay?

  Response: No. Testing the capability of labs to arrive at one identical MTD is not a test of the capability of the pubertal assays to perform correctly.

Issues and responses (2)

- Effect of non-endocrine-caused body weight loss on endocrine endpoints

  Issue: Do the pubertal assays inappropriately yield positive results due simply to body-weight changes?

  Response: No. A restricted-feeding study done by EPA's ORD showed that there is no interference from simple body-weight loss as long as the difference is no greater than 10% of the control values.

Issues and responses (3)

- Thyroid activity

  Issue: Not enough thyroid chemicals have been tested in the pubertals.

  Response: EPA added thyroid chemicals to the prevalidation and validation studies. EPA's ORD also did pubertal assays on several thyroid-active compounds that work via different mechanisms.

Issues and responses (4)

- Rat strain sensitivity

  Issue: Is EPA using the most sensitive rat strain for the pubertals?

  Response: There seems to be no practical alternative that will maximize sensitivity for all endpoints in the pubertal assay. Most of the validation work used the Sprague-Dawley rat due to the amount of historical data available, but other strains have been shown to respond similarly (Long-Evans, Wistar, Alderley Park).

Issues and responses (5)

- Toxic negatives

  Issue: Do the pubertals respond to endocrine changes induced by stress from other toxicity, thus making it likely that all chemicals will test positive at MTD?

  Response: There are few if any chemicals which have been tested for all modes of endocrine activity and proven to be negative in all. EPA has found two chemicals in males and one in females which have been shown not to have effects on pubertal assay endpoints despite significant body weight loss that did not exceed 10%.

Issues and responses (6)

- Phytoestrogens in feed

  Issue: Should low-phytoestrogen feed be required for the pubertal assays?

  Response: No. There is no indication that the presence of phytoestrogens in feed is a sensitivity-limiting factor for the pubertal assays at levels below about 350 ug/g. Capping the level should be sufficient.

Current study – interlab validation

- 3 contract labs
- 3 chemicals (female), 4 chemicals (male)
- Test all endpoints, not all modes of action
- Compare results across labs, by chemical, to see if responses are consistent.
- Effort began in July 2004; in-life completed by April 2005, draft reports received Sept 2005.
- Interlaboratory statistical comparison being done via a uniform analysis.

Plans for next EDMVAC meeting

- Present data from the interlaboratory validation study
- Review the entire case for validation of the pubertals, including studies done outside of the EPA efforts since the Crit Rev Toxicol review.